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The role of small molecule enhancers on the catalytic efficiency of HLA-DM

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MS is the only neurological disorder that has a large body of evidence to support that it has an autoimmune basis. The diverse pathogenic mechanisms of autoimmune diseases are not well understood and different hypothetical models exist. Experimental autoimmune encephalomyelitis is an inflammatory demyelinating disease that serves as a mouse model for Multiple Sclerosis. Activated CD4 T cells that recognize CNS autoantigens mediate EAE and are thought to have a central role in MS pathogenesis (89).

CD4 T cell activation is dependent on the process of antigen presenting as previously described. DM plays a critical role in antigen presenting to CD4 T cells by catalyzing the exchange of peptides bound to MHC class II molecules. Mice that are deficient in DM show to be resistant to the induction of EAE and disease cannot be induced by immunization with intact myelin oligodendrocyte protein or even by transfer of specific T cells (55). In summary these considerations support the notion that DM plays a decisive role in the disease progress and the regulation of DM-catalyzed peptide loading represents a potential therapeutic approach in the treatment of MS.

In order to gain insight into these mechanisms we performed High-throughput screening and identified four small molecules that substantially accelerate the rate of DM catalyzed peptide exchange. The data demonstrated that these small molecules do not change the equilibrium of the reaction but significantly shortened the time required to reach it. For all enhancers, no plateau was observed even at relatively high concentrations of these compounds indicating that they induce a striking acceleration of the reaction despite a low affinity. The four structurally distinct compounds fall into two functional classes; two compounds are only active in the presence of DM, and microdialysis experiments show a direct interaction with DM. The remaining two compounds have partial activity in the absence of DM, suggesting that they may act at the interface between DM and DR2/peptide. All small molecules had striking effects on the Vmax and the $Vmax/K_M$, but little or no effect on the K_M, demonstrating that the small molecules modulate the efficiency of catalysis rather than early

steps in the enzyme substrate interaction because substantial changes in the affinity would be expected to modify the K_M. The four small molecules share this functional property even though they are structurally quite diverse and may promote different facets of the conformational changes within the DM:DR2/peptide complex that lead to peptide release. Department of medicinal chemistry was employed to identify regions of M19 that were important for activity (Figure 27). Our attempt to define the binding site of the enhancers involved x-ray crystallography. The structure of the soaked crystals were solved but unambiguous density representing bound enhancers could not be identified. However, this yielded a more complete structure of DM compared to the deposited Protein Data Bank (PDB). Melissa Nicholson investigated this area as a potential binding site for the four enhancers and could show that hydrophobic ridge in the DMβ1 domain was implicated in the catalysis of peptide exchange. These results demonstrated that the catalytic efficiency of DM can be substantially increased by several small molecule enhancers, raising the possibility that the editing function of DM could be increased to modify the peptide repertoire presented to CD4 T cells. Biasing the repertoire toward increased representation of high-affinity peptides may be useful in the induction of antimicrobial or antitumor T cell responses mediated by T cells that recognize peptides bound with high-affinity to MHC class II molecules. Self-peptides with an intermediate or low affinity have been implicated in animal models of T cell mediated autoimmune diseases e.g. EAE, and presentation of such peptides may be reduced when the editing function of DM in increased (114). The compounds that are currently available have limited solubility and also have shown toxicity in preliminary cellular studies, indicating that they need to be modified for evaluation of these potential applications. The small molecules may also be valuable in conjunction with structural studies for further elucidating the mechanism by which DM catalyzes peptide exchange.